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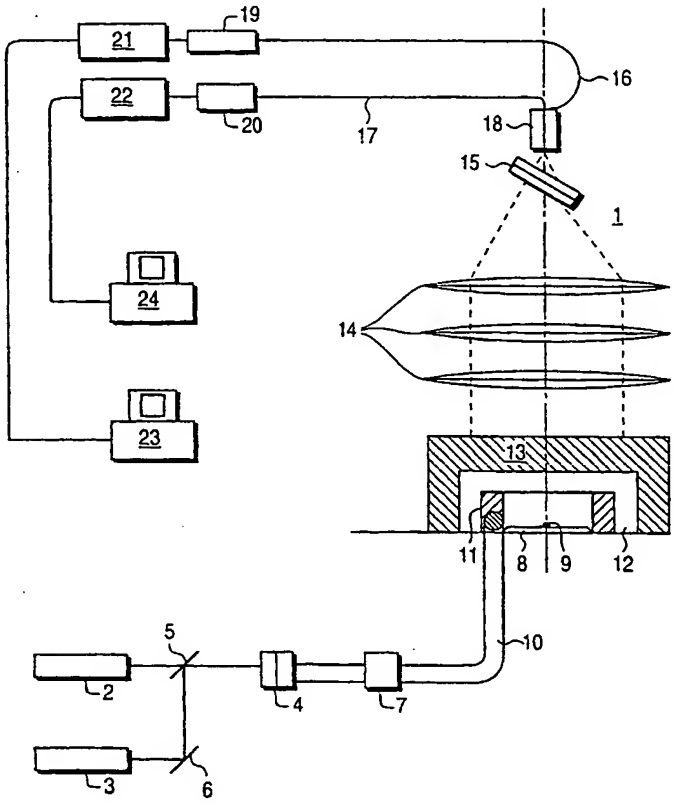

(57) Abstract: The device comprises two light sources (2, 3), a band pass filter (4), mirrors (5, 6), polarizer (7), substrate (8), optical fiber (10) for transmitting optical energy from the light sources (2, 3) to microcavity (11), signal analyzer (21) and data processor (23).

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ADDRESSABLE ARRAYS USING MORPHOLOGY DEPENDENT RESONANCE FOR ANALYTE DETECTION

Related Applications

This application claims priority to United States Provisional Patent Application, titled "Nanoparticle Structures With Receptors for Raman Spectroscopy," inventors: David I. Kreimer, Ph.D., Oleg A. Yevin, Ph.D., Thomas H. Nufert, filing date: September 27, 1999, Serial No: 60/156,195, to United States Provisional Patent Application, titled "Addressable Arrays Using Morphology Dependent Resonance for Analyte Detection," inventors Oleg A. Yevin, Ph.D., David I. Kreimer, Ph.D., filing date September 27, 1999, Serial No. 60/156,145 and to United States Provisional Patent Application titled "Fractal Absorber for Heat Pipes with Broad Range Heat Radiation Absorptivity", inventors, Oleg Yevin, Thomas H. Nufert and David I. Kreimer, Serial No. 60/156,471 . Each of these Provisional Patent Applications is herein incorporated fully by reference.

BACKGROUND OF THE INVENTION

Field of the Invention

This invention relates to methods for the manufacture and use of devices for detection of analytes. Specifically, the invention relates to methods and devices for the detection of analytes associated with a microobject to create morphology dependent resonance conditions. More specifically, the invention relates to methods and devices for separating analytes from other molecules upon binding of an analyte to a specifically prepared surface, including fractal surfaces, and placing an analyte in an area suitable for its detection using Raman, fluorescence or other methods for spectroscopy.

Description of Related Art

The detection of analytes is an important aspect of current biology, biotechnology, chemistry, and environmental industries. Detection of analytes can be accomplished using many different methods, including the chemical methods of chromatography and mass spectroscopy. Other methods for detecting analytes depend upon specific binding of analytes or "ligands" to other molecules, herein termed "receptors."

I. Detection of Analytes

The detection of analyte, or "ligand" molecules is an important aspect of current biology, biotechnology, chemistry, and environmental industries. Detection of ligands can be accomplished using many different methods, including the chemical methods of chromatography, mass spectroscopy, nucleic acid hybridization and immunology. Hybridization and immunological methods rely upon the specific binding of ligands to detector, or "receptor" molecules. The basis for specificity of these methods is conferred by a receptor molecule can bind in a specific fashion to the ligand molecule, thereby creating a bound complex. Upon treating the complex under conditions that favor the removal of unbound ligand, the bound ligand can be assayed. The specificity of the binding, the completeness of separating bound and unbound ligands and receptors, and the sensitivity of the detection of the ligand confers the selectivity of the detection system.

For example, in biology and biotechnology industries, analytes such as deoxyribonucleic acid ("DNA") and messenger ribonucleic acid ("mRNA") are important indicators of specific genetic, physiological or pathological conditions. DNA can contain important information about the genetic makeup of an organism, and mRNA can be an important indicator of which genes are active in a specific physiological or pathological condition and what proteins may be

created as a result of gene activation. Additionally, the direct detection of proteins can be important to the understanding of the physiological or pathological condition of an individual.

DNA is made of a double helix of two strands, each of which is composed of a series or "sequence" of nucleotide bases. The bases found in DNA include adenine, thymine, cytosine and guanine. One strand of the double helix has a sequence of the nucleotides that can be transcribed into mRNA, herein termed a "reading strand," and the other strand has a sequence of bases, each of which is complementary to the base in the position corresponding in the reading strand. For every adenine in the reading strand, a thymine is present in the other strand. Similarly, for every cytosine in the reading strand, a guanine is present in the other strand. For every guanine and adenine in the reading strand, a cytosine and a thymine, respectively, is found in the other strand. Thus, when the two strands are aligned properly with respect to the other, the complementary bases of each strand can form hydrogen bonds, thereby holding the two strands in a complex, or "hybrid" according to the model of Watson and Crick ("Watson-Crick" hybridization). Thus, the two strands are considered herein to be "complementary" to each other. Ribonucleic acid has a similar structure as DNA, except that thymine is typically replaced by the base uracil. However, uracil is complementary to adenine, and thus, hybridization of RNA can occur with DNA. Because the information content of nucleic acids resides significantly in the sequence of the units that make up the nucleic acid, purely chemical methods that can detect only the presence of nucleotide bases are of limited usefulness. Thus, methods for detecting the presence of specific DNA or RNA relies upon the characterization of the sequence of bases of that nucleic acid.

Many different methods are currently in use for the detection of nucleic acids and proteins, but those methods can be time-consuming, expensive, or

poorly reproducible. For example, the detection of specific nucleic acid sequences in DNA or RNA molecules can be accomplished using hybridization reactions, wherein an analyte DNA or RNA molecule is permitted to attach to a complementary sequence of DNA. A complementary DNA molecule can be attached to a supporting matrix, and the bound DNA and matrix is herein termed a "substrate." Exposing an analyte nucleic acid to a complementary substrate DNA can result in the formation of a relatively stable hybrid. Detection of the duplex DNA hybrid is characteristically carried out using methods that can detect labeled DNA analytes. The labeling is typically performed using radioactive, spin resonance, chromogenic or other labels, which are attached to the analyte molecules. Thus, when the labeled analyte attaches to the substrate, unbound analyte can be removed and the bound, or specific, analyte can be detected and quantified.

For example, to detect a mRNA molecule having a specific sequence using current methods, naturally occurring, or "native" mRNA is typically converted to a complementary DNA ("cDNA") molecule using an enzyme called "reverse transcriptase" under conditions that incorporate a labeled nucleotide into the cDNA. Upon binding of the labeled cDNA to the hybridization substrate, the bound ligand can be detected using a radiometric technique such as scintillation counting, fluorescence or spin resonance, depending on the type of label used.

Currently available methods for the detection of nucleic acids and proteins have undesirable characteristics. The methods are time consuming, require expensive equipment and reagents, require expert manual operations, and the reagents can be environmentally hazardous. Additionally, for assaying mRNA, the methods also can be sensitive to defects in the fidelity of reverse transcription. Unless the cDNA made during reverse transcription is exactly complementary to the mRNA, the analyte will not have the same sequence as the

native mRNA, and misleading results can be obtained. The amplification of nucleic acid sequences by the polymerase chain reaction ("PCR") has been used to increase the numbers of nucleic acid molecules (complementary DNA or "cDNA") that can be detected. PCR requires DNA polymerase enzymes to amplify the cDNA. Some DNA polymerases can insert incorrect bases into a growing strand of newly synthesized cDNA. In addition, the recognition of ceratin cDNA by DNA polymerase and primers used for PCR can vary depending on the specific sequences of DNA in the sample to be amplified. This variation can result in non-proportional amplification of different cDNA molecules. Subsequent amplification of an strand having an incorrect sequence can result in the presence of several different cDNA sequences in the same sample. Thus, the accuracy and sensitivity of analysis of cDNA using PCR can be compromised.

Additionally, for medical diagnostic or forensic purposes, it can be very important for results of tests to be available rapidly. Commonly used methods for detection of specific nucleic acid sequences can be too slow for therapeutic or forensic uses. Thus, there is a need for rapid, accurate measurement of nucleic acid sequences.

II. Fluorescence Spectroscopy

When an analyte in question specifically binds to a corresponding antibody is capable of generating a distinctive fluorescent signal, no labeling is required. However, such intrinsic fluorescence is too weak or is absent, and thus, these methods cannot be used. Fluorescence labels of various types are commercially available and can permit the use of fluorescence detection.

III. Raman Spectroscopy

Raman spectroscopy involves the use of electromagnetic radiation to generate a signal in an analyte molecule. Raman spectroscopic methods have only recently been developed to the point where necessary sensitivity is possible. Raman spectroscopic methods and some ways of increasing the sensitivity of Raman spectroscopy are described herein below.

A. Raman Scattering

According to a theory of Raman scattering, when incident photons having wavelengths in the near infrared, visible or ultraviolet range illuminate a certain molecule, a photon of that incident light can be scattered by the molecule, thereby altering the vibrational state of the molecule to a higher or a lower level. The vibrational state of a molecule is characterized by a certain type of stretching, bending, or flexing of the molecular bonds. The molecule can then spontaneously return to its original vibrational state. When the molecule returns to its original vibrational state, it can emit a characteristic photon having the same wavelength as the incident photon. The photon can be emitted in any direction relative to the molecule. This phenomenon is termed "Raleigh Light Scattering."

A molecule having an altered vibrational state can return to a vibrational state different from the original state after emission of a photon. If a molecule returns to a state different from the original state, the emitted photon can have a wavelength different from that of the incident light. This type of emission is known as "Raman Scattering" named after C. V. Raman, the discoverer of this effect. If, a molecule returns to a higher vibrational level than the original vibrational state, the energy of the emitted photon will be lower (i.e., have longer wavelength) than the wavelength of the incident photon. This type of Raman scattering is termed "Stokes-shifted Raman scattering." Conversely, if a

molecule is in a higher vibrational state, upon return to the original vibrational state, the emitted photon has a lower energy (i.e., have a shorter wavelength). This type of Raman scattering is termed "anti-Stokes-shifted Raman scattering." Because many more molecules are in the original state than in an elevated vibrational energy state, typically the Stokes-shifted Raman scattering will predominate over the anti-Stokes-shifted Raman scattering. As a result, the typical shifts of wavelength observed in Raman spectroscopy are to longer wavelengths. Both Stokes and anti-Stokes shifts can be quantitized using a Raman spectrometer.

B. Resonance Raman Scattering

When the wavelength of the incident light is at or near the frequency of maximum absorption for that molecule, absorption of a photon can elevate both the electrical and vibrational states of the molecule. The efficiency of Raman scattering of these wavelengths can be increased by as much as about 1000 times the efficiency of wavelengths substantially below the absorption maximum. Therefore, upon emission of the photon with return to the ground electrical state, the intensity of Raman scattering can be increased by a similar factor. This phenomenon is herein termed "Resonance Raman Scattering."

C. Surface Enhanced Raman Scattering

When Raman active molecules are excited near to certain types of metal surfaces, a significant increase in the intensity of the Raman scattering can be observed. The increased Raman scattering observed at these wavelengths is herein termed "Surface Enhanced Raman scattering." The metal surfaces that exhibit the largest increase in Raman intensity comprise minute or nanoscale rough surfaces, typically coated with minute metal particles. For example, nanoscale particles such as metal colloids can increase intensity of Raman

scattering to about 10^6 times or greater, than the intensity of Raman scattering in the absence of metal particles. This effect of increased intensity of Raman scattering is termed "Surface Enhanced Raman scattering" or "SERS."

The mechanism of SERS is not known with certainty, but one factor can affect the enhancement. Electrons can typically exhibit a vibrational motion, termed herein "plasmon" vibration. Particles having diameters of about 1/10th the wavelength of the incident light can contribute to the effect. Incident photons can induce a field across the particles, and thereby can alter the movement of mobile electrons in the metal. As the incident light cycles through its wavelength, the induced motion of electrons can follow the light cycles, thereby creating an oscillation of the electron within the metal surface having the same frequency as the incident light. The electrons' motion can produce a mobile electrical dipole within the metal particle. When the metal particles have certain configurations, incident light can cause groups of surface electrons to oscillate in a coordinated fashion, thereby causing constructive interference of the electrical field so generated, creating an area herein termed a "resonant domain." The enhanced electric field due to such resonance domains therefore can increase the intensity of Raman scattering and thereby can increase the intensity of the signal detected by a Raman spectrometer.

The combined effects of surface enhancement and resonance on Raman scattering is termed "Surface Enhanced Resonance Raman scattering" or "SERRS." The combined effect of SERRS can increase the intensity of Raman scattering by about 10^{14} or more. It should be noted that the above theories for enhanced Raman scattering may not be the only theories to account for the effect. Other theories may account for the increased intensity of Raman scattering under these conditions.

D. Raman Methods for Detection of Nucleic Acids and Proteins

Several methods have been used for the detection of nucleic acids and proteins. Typically, an analyte molecule can have a reporter group added to it to increase the ability of an analytical method to detect that molecule. Reporter groups can be radioactive, flourescent, spin labeled, and can be incorporated into the analyte during synthesis. For example, reporter groups can be introduced into cDNA made from mRNA by synthesizing the DNA from precursors containing the reporter groups of interest. Additionally, other types of labels, such as rhodamine or ethidium bromide can intercalate between strands of bound nucleic acids in the assay and serve as reporter groups of hybridized nucleic acid oligomers.

In addition to the above methods, several methods have been used to detect nucleic acids using Raman spectroscopy. Vo-Dinh, U.S. Patent No: 5,814,516; Vo-Dinh, U.S. Patent No: 5,783,389; Vo-Dinh, U.S. Patent No: 5,721,102; Vo-Dinh, U.S. Patent No: 5,306,403. These patents are herein incorporated fully by reference. Recently, Raman spectroscopy has been used to detect proteins. Tarcha et al., U.S. Patent No: 5,266,498; Tarcha et al., U.S. Patent No: 5,567,628, both incorporated herein fully by reference, provide an analyte that has been labeled using a Raman active label and an unlabeled analyte in the test mixture. The above-described methods rely upon the introduction of a Raman active label, or "reporter" group, into the analyte molecule. The reporter group is selected to provide a Raman signal that is used to detect and quantify the presence of the analyte.

By requiring reporter groups to be introduced into the analyte, additional steps and time are required. Additionally, the above methods can require extensive washing of the bound and unbound Raman labeled analytes to provide the selectivity and sensitivity of the assay. Moreover, because specific Raman labels must be provided for each type of assay system used, properties of the

analytes must be determined in advance of the assay.

IV. Detection of Proteins and Low Molecular Weight Analytes Binding to Receptor Molecules

Detection of proteins and low molecular analytes is based upon either various techniques aimed at separation of these analytes and detection of purified analytes, or upon detection utilizing specific agents capable of highly specific recognition of such targets. The techniques based upon separation are laborious, expensive and require highly trained personnel. In addition, unstable proteins can make purification procedures difficult. The recognition is often achieved by the use of highly specific proteins called antibodies. However, antibodies, protein molecules capable of tight and selective binding of analytes are available commercially or can be generated as upon request by several companies.

If, prior to formation of a complex with an analyte in question, a corresponding antibody is attached to a substrate, the analyte bound to such immobilized antibody can be retained on such substrate. This is a basis for a large number of immunoassays for detection of various analytes, such as proteins, peptides, sugars, low molecular weight compounds of organic origin, and even novel, chemical compounds synthesized in vitro that may not be present in nature. One characteristic of these conventional immunoassays is a necessity to have an analyte somehow labeled to be detected. Typically labels utilize distinctive fluorescence, radioactivity, or enzymatic activity. These signals are introduced using other antibodies or by chemical modification of the analyte molecule. These, additional procedures can often be non-linear and therefore can be cumbersome and difficult to interpret. These problems can be addressed by increasing the sensitivity of the detection and/or by developing approaches which would exclude the introduction of labels.

V. Morphology Dependent Resonance (MDR)

Optical signals can be also enhanced in cylindrical or spherical microcavities, hollow tubes or other optical resonators. According to one theory, this enhancement occurs due to resonances resulting from confinement of the radiation within these resonators. Particularly attractive for the purposes of the enhancement of analytes' optical signals are microcavities or other microobjects, which allow for ether embracing an analyte inside or placing it outside on their surfaces. These microobjects and microcavities, in fact, can vary in size from a few microns up to a few centimeters in diameter. The resonances in such dielectric systems, called morphology dependent resonances ("MDRs"), can occur due to total internal reflection, which can result in the accumulation of light within or on the surface of such microobjects. The accumulation can lead to increasing the intensity of incident light. The emitted light can also accumulate under these conditions. Thus, the signal of analytes can be enhanced. However, the methods and devices currently available for MDR-enhancement are not sufficiently easy to use.

VI. Arrays

When several kinds of immobilized receptor molecules, each designed for a correspondent analyte and attached at a correspondent place, are utilized simultaneously, the system of such receptors forms an array. Such arrays allow for simultaneous detection of several analyte. Most developed are DNA arrays (DNA chips) utilizing high affinity between two complementary oligonucleotides (see above). Proteins are also capable of high affinity to each other, oligonucleotides and to small substrate molecules (see above). Thus, selective binding of proteins, oligonucleotides or other cellular constituents to their respective high-affinity target molecules is achievable, and such receptor molecules can be arranged in an array for parallel analysis of these heterogeneous

analytes. Such parallel analysis of heterogeneous analytes is of great importance for comprehensive biochemical characterization of normal biological processes and diseases, including viral and bacterial infections.

Arrays are typically organized by placing target molecules in a known spatial order on a matrix plain. Alternatively, target molecules can be attached to a mobile matrix elements (beads), which upon binding of respective analyte, are physically separated and identified by the color of beads (Michael KL, Taylor LC, Schultz SL, Walt DR *Randomly ordered addressable high-density optical sensor arrays.* Analytical Chemistry 70:1242-1248 (1998), incorporated herein fully by reference). Detection of bound analytes is generally performed utilizing a unique property of analyte, such as its radioactivity, fluorescence, ability to stimulate a specific chemical reaction and so on. Some of these approaches, although useful for detection of a single kind of analyte, are in general inapplicable for parallel detection of heterogeneous analytes.

When bound species are fluorescence labeled, simultaneous reading of the results of binding of analytes on arrays is highly desirable. Large number of various receptors (tens of thousands) placed on modern arrays can make one-by-one reading unacceptably long. Reading of fluorescence signal from cell units of such array is possible with CCD (charge-coupled device), which allows to make an image of fluorescence signal coming from an array. The problem however is that weak signals can be detected only if the signal-to-noise ratio of the image is high. Noise arises from fluorescence scattered from adjacent samples.

SUMMARY OF THE INVENTION

Thus, an object of this invention is the development of Raman, fluorescence and other methods for detecting electromagnetic signals generated

by analytes that utilize the advantages of MDR enhancement for efficient detection.

Another object of this invention is the development of methods for manufacturing and the manufacture of arrays for analytes detection conjugated with amplifiers.

Yet another object of this invention is the development of methods for manufacturing and the manufacture of arrays for heterogeneous analytes utilizing detection which do not require analyte labeling or distinctive spectral differences to exist between the analyte and receptor.

An additional object of this invention is the development of methods for manufacturing and the manufacture of devices which allow the detection of analytes under MDR conditions and achieving optical alignment in dielectric microobjects loaded with analytes.

A further object of this invention is the development of methods for manufacturing and the manufacture of devices which allow to readily read the results of binding by means of parallel reading, while improving signal-to-noise ratio.

These and other objects are met by the design and manufacture of devices incorporating an analyte and a cavity or microobject providing morphology dependent resonance conditions (MDR conditions) combined with a detector of electromagnetic radiation, including those characteristic of Raman, infrared, ultraviolet, fluorescent and other assays.. Microobjects can have a variety of different shapes and configurations, including but not limited to spheres, cylinders, hollow tubes, other structures having curved cross sections, prisms, hexagons and other higher regular polygons. Conical structures can provide additional enhancement of a broad range of spectral features from analytes. The mobile character of these three principal components (an analyte, a microobject and detector) involved in this conjugation allows for the resolution

of two principal steps: placing the analyte under MDR conditions and measuring of the signal from the analyte. Conjugation eliminates the problem of optical mis-alignment, allows for simplified, less expensive instrumentation, requires less power and improves signal-to-noise ratio. At the same time, aspects of this invention can dramatically increase optical signals from analytes.

In certain embodiments of this invention, desirable conjugations can be archived by placing a receptor for a corresponding analyte onto the surface of a bead, and this bead is then placed into a MDR-microwell combined with optical fiber or waveguide for signal transduction. In other embodiments, the beads can have a particle structure on its surface that provide enhanced resonance. In still additional embodiments, particle structures can be placed within microcavities. In yet other embodiments, an array of receptor molecules on a planar or non-planar support, upon binding of correspondent analytes, can be placed into a microcavity, or alternatively, a system of microcavities can be placed onto the surface of such support.

In other embodiments, the methods are developed to avoid the problem of noise arising from optical signal scattered from adjacent samples. This is achieved by embracing or holding the sample in an individual microwell and collecting the light signal using individual optic path. Alternatively, non-planar arrangement of array can be employed to place a whole set of analytes on such an array under MDR conditions, and wherein optical fibers are placed into close proximity to sample compartments of this array, thus eliminating light generated in one compartment from being detected erroneously in a nearby compartment (herein termed a "parasite" signal).

By using conjugated systems, the collection of light signal from each sample compartment in parallel can be achieved. This way of collecting signals can allow for convenient direction of signals from all sample compartments simultaneously or in a defined order to a photo-detector(s) such as CCD devices,

photo-diodes, photomultiplier tubes ("PMT"). The instrumentation for such analysis does not require special optical alignment, because the signal transmitter is made of optical fibers or waveguides precast to collect light directly from the sample.

BRIEF DESCRIPTION OF THE FIGURES

Figure 1 is a schematic diagram of a system of this invention for combined fluorescence and Raman detection of binding on a substrate.

Figure 2 shows an "external" optical collecting system of this invention with a cavity on the tip of an optic fiber.

Figure 3 shows the "inner" optical collecting system of this invention.

Figures 4a - 4d show different embodiments of analyte compartment arrangement in the array detection system of this invention.

Figures 5a - 5c shows different types of arrangements for a low density array of this invention.

Figures 6a - 6c illustrate the embracement of various biochips into a microcavity of this invention.

Figures 7a - 7c show MDR devices of this invention incorporating conical optic fibers for transmitting a signal from bioanalytes to a detector.

Figures 8a a- 8b show an arrangement for randomly addressable arrays of this invention.

Figures 9a - 9c show an arrangement for randomly addressable arrays with an external light transmitter of this invention.

Figures 10a - 10b show different types of high density, randomly addressable arrays of this invention placed inside a microcavity.

Figures 11a - 11b show a non-planar array of this invention as arranged as a cylinder.

Figure 12 shows a non-planar array of this invention inside microcavity.

Figures 13a - 13b show a disposable non-planar (microcylindric) array of this invention inside a cylindrical light transmitter system.

Figures 14a - 14b show a microcylindrical array of this invention.

DETAILED DESCRIPTION OF THE INVENTION

Definitions

The following words and terms are used herein.

The term "analyte" as used herein means molecules, particles or other material whose presence and/or amount is to be determined. Examples of analytes include but are not limited to deoxyribonucleic acid ("DNA"), ribonucleic acid ("RNA"), amino acids, proteins, peptides, sugars, lipids, glycoproteins, cells, sub-cellular organelles, aggregations of cells, and other materials of biological interest.

The term "defined area" or "cell" as used herein means a location in an array that is capable of independent manufacture or detection. A defined area may be identified positionally or by use of a specific label of that region, such as a polymer or a bead having a characteristic feature.

The term "fractal" as used herein means a structure comprised of elements, and having a relationship between the scale of observation and the number of elements, i.e., scale-invariant. By way of illustration only, a continuous line is a 1-dimensional object. A plane is a two-dimensional object and a volume is a three-dimensional object. However, if a line has gaps therein, and is not a continuous line, the dimension is less than one. For example, if ½ of the line is missing, then the fractal dimension is ½. Similarly, if points on a plane are missing, the fractal dimension of the plane is between one and 2. If ½ of the points on the plane are missing, the fractal dimension is 1.5. Moreover, if ½ of the points of a solid are missing, the fractal dimension is 2.5. In scale invariant structures, the structure of objects appears to be similar, regardless of the size of

the area observed. Thus, fractal structures are a type of ordered structures, as distinguished from random structures, which are not ordered.

The term "fractal associate" as used herein, means a structure of limited size, comprising at least about 100 individual particles associated together, and which demonstrates scale invariance within an area of observation limited on the lower bound by the size of the individual particles comprising the fractal associate and on the upper bound by the size of the fractal associate.

The term "fractal dimension" as used herein, means the exponent D of the following equation: $N \propto R^{D}$, where R is the area of observation, N is the number of particles, and D is the fractal dimension. Thus in a non-fractal solid, if the radius of observation increases by 2-fold, the number of particles observed within the volume increases by 2^3. However, in a corresponding fractal, if the radius of observation increases by 2-fold, the number of particles observed increases by less than 2^3.

The term "fractal particle associates" as used herein means a large number of particles arranged so that the number of particles per unit volume (the dependent variable) or per surface unit changes non-linearly with the scale of observation (the independent variable).

The term "label" as used herein means a moiety having a physicochemical characteristic distinct from that of other moieties that permit determination of the presence and/or amount of an analyte of which the label is a part. Examples of labels include but are not limited to fluorescence, spin-resonance, radioactive moieties. Also known as reporter group.

The term "linker" as used herein means an atom, molecule, moiety or molecular complex having two or more chemical groups capable of binding to a surface and permitting the attachment of particles together to form groups of particles. The simplest linker connects two particles. A branched linker may link together larger numbers of particles.

The term "microobject", "microcavity" or "MDR amplifier" as used herein, mean a cavity providing multiple passes of a light beam to provide multiple interactions of that light beam with an analyte moiety. In general, a microobject or microcavity has an average dimension in the range of about 3 λ to about 4 cm.

The term "morphology dependent resonance" or "MDR" as used herein, means a situation wherein a beam of light has multiple interactions with an analyte moiety to increase the magnitude of an electromagnetic signal generated by the interactions of the light beam with the analyte.

The term "ordered structures" as used herein means structures that are non-random.

The term "particle structures" as used herein means a group of individual particles that are associated with each other in such a fashion as to permit enhancement of electric fields in response to incident electromagnetic radiation. Examples of particles include metals, metal-coated polymers and fullerenes. Also included in the meaning of the term "particle structures" are films or composites comprising particles on a dielectric surface or imbedded in a dielectric material.

The term "percolation point" as used herein means a point in time on a conductive surface or medium when the surface exhibits an increase in conductance, as measured either via surface or bulk conductance in the medium. One way to measure surface or "sheet" conductance is via electric probes applied to the surface.

The term "Raman array reader" as used herein means a device having a light source and a light detector.

The term "Raman signal" as used herein means a Raman spectrum or portion of Raman spectrum.

The term "Raman spectral feature" as used herein means a value obtained as a result of analysis of a Raman spectrum produced for an analyte under conditions of detection. Raman spectral features include, but are not limited to, Raman band frequency, Raman band intensity, Raman band width, a ratio of band widths, a ratio of band intensities, and/or combinations the above.

The term "Raman spectroscopy" as used herein means a method for determining the relationship between intensity of scattered electromagnetic radiation as a function of the frequency of that electromagnetic radiation.

The term "Raman spectrum" as used herein means the relationship between the intensity of scattered electromagnetic radiation as a function of the frequency of that radiation.

The term "random structures" as used herein means structures that are neither ordered nor fractal. Random structures appear uniform regardless of the point and scale of observation, wherein the scale of observation encompasses at least a few particles.

The term "receptor" as used herein means a moiety that can bind to or can retain an analyte under conditions of detection.

The term "resonance" as used herein means an interaction with either incident, scattered and/or emitted electromagnetic radiation and a surface having electrons that can be excited by the electromagnetic radiation and increase the strength of the electric field of the electromagnetic radiation.

The term "resonance domain" as used herein means an area within or in proximity to a particle structure in which an increase in the electric field of incident electromagnetic radiation occurs.

The term "reporter group" as used herein means label.

The term "reverse Raman spectroscopy" ("RRS") as used herein means an application of Raman spectroscopy in which an analyte is distinguished by the

presence of a Raman spectral feature that is not found in a receptor for that analyte or in the medium in which the analysis is performed.

The term "scaling diameter" as used herein means a relationship between particles in a nested structure, wherein there is a ratio (scaling ratio) of particle diameters that is the same, regardless of the size of the particles.

The term "surface enhanced Raman spectroscopy" ("SERS") as used herein means an application of Raman spectroscopy in which intensity of Raman scattering is enhanced in the presence of an enhancing surface.

The term "surface enhanced resonance Raman spectroscopy" ("SERRS") as used herein means an application of Raman spectroscopy in which Raman signals of an analyte are enhanced in the presence of an enhancing surface (see SERS) and when an absorption band of the analyte overlaps with the wavelength of incident electromagnetic radiation.

Embodiments of The Invention

Certain embodiments of the devices and methods of this invention are based upon the conjugation of a compartment containing an analyte with a microobject providing morphology dependent resonances conditions (MDR conditions) combined with a signal transmitter. The mobile character of these three principal components (an analyte compartment, a microobject and signal transmitter) involved in this conjugation allows for the resolution of two principal steps, placing the analyte under morphology dependent resonance conditions and measurements of the signal from the analyte. The invention also includes methods for detecting analytes using its optical properties.

The methods and compositions of this invention represent improvements over the existing methods for spectroscopic methods for detection and quantification of analyte molecules. In particular, the compositions and methods can be desirable for use in conjunction with infrared spectroscopy, fluorescence

spectroscopy, surface plasmon resonance, Raman spectroscopy, mass spectroscopy or any other method utilizing excitation of an analyte by electromagnetic radiation.

Certain embodiments of this invention are based upon Surface Enhanced Raman Spectroscopy ("SERS"), Surface Enhanced Resonance Raman Spectroscopy ("SERRS") and Reverse Raman Spectroscopy ("RRS"). This invention includes methods for manufacturing Raman active structures having specific analyte receptor molecules attached to those structures. The invention also includes methods for detecting analytes using Raman spectroscopy, reverse Raman spectroscopy, compositions useful for reverse Raman spectroscopy, and arrays and test kits embodying Raman spectroscopic methods.

The structures that are desirable for use according to the methods of this invention include structures of small particles in structures, herein termed particle structures, which includes as a subset, fractal associates. Particle structures can be characterized by having physical and chemical structures that enable oscillations of electrons to be in resonance with incident and outgoing electromagnetic radiation.

I. Manufacture of Particle Structures

The Raman active structures desirable for use according to this invention can include any structure in which Raman signals can be amplified. The following discussion regarding metal fractal structures is not intended to be limiting to the scope of the invention, but is for purposes of illustration only.

A. Manufacture of Metal Particles

To make metal particles for nanoscale arrays of receptors according to some embodiments of this invention, we can generally use methods known in the art. Tarcha et al., U.S. Patent No: 5,567,628, incorporated herein fully by

reference. Metal colloids can be composed of noble metals, specifically, elemental gold or silver, copper, platinum, palladium and other metals known to provide surface enhancement. In general, to make a metal colloid, a dilute solution containing the metal salt is chemically reacted with a reducing agent. Reducing agents can include ascorbate, citrate, borohydride, hydrogen gas, and the like. Chemical reduction of the metal salt can produce elemental metal in solution, which combine to form a colloidal solution containing metal particles that are relatively spherical in shape.

Example 1: Manufacture of Gold Colloid and Fractal Structures

In one embodiment of this invention, a solution of gold nuclei is made by preparing a 0.01% solution of $NaAuCl_4$ in water under vigorous stirring. One milliliter ("ml") of a solution of 1% sodium citrate is added. After 1 minute of mixing, 1 ml of a solution containing 0.075 % $NaBH_4$ and 1% sodium citrate is added under vigorous stirring. The reaction is permitted to proceed for 5 minutes to prepare the gold nuclei having an average diameter of about 2 nm). The solution containing the gold nuclei can be refrigerated at 4° C until needed. This solution can be used as is, or can be used to produce particles of larger size (e.g., up to about 50 nm diameter), by rapidly adding 30 µl of the solution containing gold nuclei and 0.4 ml of a 1% sodium citrate solution to the solution of 1% $HAuCl_4{\cdot}3H_2O$ diluted in 100 ml H_2O, under vigorous stirring. The mixture is boiled for 15 minutes and is then cooled to room temperature. During cooling, the particles in the solution can form fractal structures. The resulting colloid and/or fractal particle structures can be stored in a dark bottle.

Deposition of enhancing particles on dielectric surfaces including glass can generate films that can enhance electromagnetic signals. Such films can be as thin as about 10 nm. In particular, the distribution of electric field enhancement on the surface of such a film can be uneven. Such enhancing areas

are resonance domains. Such areas can be particular useful for positioning receptors for analyte binding and detection. For films or particle structures embedded in dielectric materials, one way to manufacture enhancing structures is to treat the surface until "percolation points" appear. Methods for measuring sheet resistance and bulk resistance are well known in the art.

Example 2: Manufacture of Metal Particles and Fractal Structures Using Laser Ablation

In addition to liquid phase synthesis described above, laser ablation is used to make metal particles. A piece of metal foil is placed in a chamber containing a low concentration of a noble gas such as helium, neon, argon, xenon, or krypton. Exposure to the foil to laser light or other heat source causes evaporation of the metal atoms, which, in suspension in the chamber, can spontaneously aggregate to form fractal or other particle structures as a result of random diffusion. These methods are well known in the art.

B. Manufacture of Films Containing Particles

To manufacture substrates containing metal colloidal particles of one embodiment of this invention, the colloidal metal particles can be deposited onto quartz slides as described in Examples 1 or 2. Other films can be made that incorporate random structures or non-fractal ordered structures in similar fashions.

Example 3: Manufacture of Quartz Slides Containing Gold Fractal Structures

Quartz slides (2.5 cm x 0.8 cm x 0.1 cm) are cleaned in a mixture of HCl:HNO_3 (3:1) for several hours. The slides are then rinsed with deionized H_2O (Millipore Corporation) to a resistance of about 18 MΩ and then with

CH_3OH. Slides are then immersed for 18 hours in a solution of aminopropyltrimethoxysilane diluted 1:5 in CH_3OH. The slides are then rinsed extensively with CH_3OH (spectrophotometric grade) and deionized H_2O prior to immersion into colloidal gold solution described above. The slides are then immersed in the gold colloid solution above. During this time, the gold colloid particles can deposit and can become attached to the surface of the quartz slide. After 24 hours, colloid derivatization is complete. Once attached, the binding of colloidal gold nanocomposites to the quartz surfaces is strong and is essentially irreversible. During the procedure, ultraviolet and/or visual light absorbance spectra of such derivatized slides are used to assess the quality and reproducibility of the derivatization procedure. The manufacturing process is monitored using electron microscopy to assess the density of the colloidal coating, the distribution of gold colloid particles on the surface, and the size of the gold colloid particles.

C. Aggregation of Particles to Form Particle Structures

According to other embodiments of this invention, several methods can be used to form particle structures. It is known that metal colloids can be deposited onto surfaces, and when aggregated can form fractal structures having a fractal dimension of about 1.8. Safonov et al., *Spectral Dependence of Selective Photomodification in Fractal Aggregates of Colloidal Particles*, Physical Review Letters 80(5):1102-1105 (1998) incorporated herein fully by reference. Figure 1 depicts a particle structure suitable for use with the methods of this invention. The particles are arranged in a scale-invariant fashion, which promotes the formation of resonance domains upon illumination by laser light.

In addition to fractal structures, ordered non-fractal structures and random structures can be generated. These different types of structures can have

desirable properties for enhancing signals associated with detection of analytes using electromagnetic radiation.

To make ordered non-fractal structures, one can use, for example, chemical linkers having different lengths sequentially as described in more detail below. In addition, using linkers of the same size, one can generate ordered structures, which can be useful for certain applications.

In certain embodiments of this invention, particles can be attached together to form structures having resonance properties. In general, it can be desirable to have the particles being spheres, ellipsoids, or rods. For ellipsoidal particles, it can be desirable for the particles to have a long axis (x), another axis (y) and a third axis (z). In general, it can be desirable to have x be from about 0.05 to about 1 times the wavelength (λ) of the incident electromagnetic radiation to be used. For rods, it can be desirable for x to be less than about 4 λ, alternatively, less than about 3 λ, alternatively less than about 2 λ, in other embodiments, less than about 1λ, and in yet other embodiments, less than about $\frac{1}{2}\lambda$. The ends of the rods can be either flat, tapered, oblong, or have other shape that can promote resonance.

For two particle structures, it can be desirable for the particle pair to have an x dimension to be less than about 4 λ, alternatively, less than about 3 λ, alternatively less than about 2 λ, in other embodiments, less than about 1λ, and in yet other embodiments, less than about $\frac{1}{2}\lambda$.

For two-dimensional structures, pairs of particles, rods, rods plus particles together can be used. The arrangement of these elements can be randomly distributed, or can have a distribution density that is dependent upon the scale of observation in a non-linear fashion.

In other embodiments, rods can be linked together end-to end to form long structures that can provide enhanced resonance properties.

For three-dimensional structures, one can use regular nested particles, or chemical arrays of particles, associated either by chemical linkers in a fractal structure or in ordered, nested arrays.

In yet other embodiments, of third-order structures, a suspension of particles can be desirable. In certain of these embodiments, the suspended particles can have dimensions in the range of about ½ λ to about 1 millimeter (mm).

Using the strategies of this invention, a researcher or developer can satisfy many needs, including, but not limited to selecting the absorbance of electromagnetic radiation by particle elements, the nature of the surface selected, the number of resonance domains, the resonance properties, the wavelengths of electromagnetic radiation showing resonance enhancement, the porosity of the particle structures, and the overall structure of the particle structures, including, but not limited to the fractal dimensions of the structure(s).

1. Photoaggregation

Photoaggregation can be used to generate particle structures that have properties which can be desirable for use in Raman spectroscopy.

Irradiation of fractal metal nanocomposites by a laser pulse with an energy above a certain threshold leads to selective photomodification, a process that can result in the formation of "dichroic holes" in the absorption spectrum near the laser wavelength (Safonov et al., Physical Review Letters 80(5):1102-1105 (1998), incorporated herein fully by reference). Selective photomodification of the geometrical structure can be observed for both silver and gold colloids, polymers doped with metal aggregates, and films produced by laser evaporation of metal targets.

One theory for the formation of selective photomodification is that the localization of optical excitations in fractal structures are prevalent in random

nanocomposites. According to this theory, the localization of selective photomodification in fractals can arise because of the scale-invariant distribution of highly polarizable particles (monomers). As a result, small groups of particles having different local configurations can interact with the incident light independently of one another, and can resonate at different frequencies, generating different domains, called herein "optical modes." According to the same theory, optical modes formed by the interactions between monomers in fractal are localized in domains that can be smaller than the optical wavelength of the incident light and smaller than the size of the clusters of particles in the colloid. The frequencies of the optical modes can span a spectral range broader than the absorption bandwidth of the monomers associated with plasmon resonance at the surface. However, other theories may account for the effects of photomodification of fractal structures, and this invention is not limited to any particular theory for operability.

Photomodification of silver fractal aggregates can occur within domains as small as about 24 x 24 x 48 nm^3 (Safonov et al., Physical Review Letters 80(5):1102-1105 (1998), incorporated herein fully by reference). The energy absorbed by the fractal medium can be localized in a progressively smaller number of monomers as the laser wavelength is increased. As the energy absorbed into the resonant domains increases, the temperature at those locations can increase. At a power of 11 mJ/cm^2, light having a wavelength of 550 nm can produce a temperature of about 600 K (Safonov et al., Physical Review Letters 80(5):1102-1105 (1998), incorporated herein fully by reference). At this temperature, which is about one-half the melting temperature of silver, sintering of the colloids can occur (Safonov et al., Id.) incorporated herein fully by reference), thereby forming stable fractal nanocomposites.

As used in this invention, photoaggregation can be accomplished by exposing a metal colloid on a surface to pulses of incident light having a

wavelengths in the range of about 400 nm to about 2000 nm. In alternative embodiments, the wavelength can be in the range of about 450 nm to about 1079 nm. The intensity of the incident light can be in the range of about 5 mJ/cm^2 to about 20 mJ/cm^2. In an alternative embodiment, the incident light can have a wavelength of 1079 nm at an intensity of 11 mJ/cm^2.

Fractal aggregates that are especially useful for the present invention can be made from metal particles having dimensions in the range of about 10 nm to about 100 nm in diameter, and in alternative embodiments, about 50 nm in diameter. A typical fractal structure of this invention is composed of up to about 1000 particles, and an area of the aggregate typically used for large-scale arrays can have a size of about 100 μm x 100 μm.

Figure 2 depicts a particle structure that have been photoaggregated and that are suitable for use with the methods of this invention. Local areas of fusion of the metal particles can be observed (circles).

2. Chemically Directed Synthesis of Particle Structures

In certain embodiments of this invention, particle structures can be made using chemical methods. First, metal particles can be either made according to methods described above, or alternatively can be purchased from commercial suppliers (NanoGram Inc., Fremont, California). Second, the particles can be joined together to form first-order structures, for example, pairs of particles. Then, the first-order structures can be joined together to form second-order structures, for example, pairs of particle pairs. Finally, third-order fractal structures can be made by joining second-order structures together.

In alternative embodiments of this invention, the formation of a fractal array of metal particles can be carried out using chemical methods. Once metal colloid particles have been manufactured, each particle can be attached to a linker molecule via a thiol or other type of suitable chemical bond. The linker

molecules then can be attached to one another to link adjacent colloid particles together. The distance between the particles is a function of the total lengths of the linker molecules. It can be desired to select a stoichiometric ratio of particles to linker molecules. If too few linker molecules are used, then the array of particles will be too loose or may not form at all. Conversely, if the ratio of linker molecules to particles is too high, the array may become too tight, and may even tend to form crystalline structures, which are not random, and therefore will not tend to promote surface enhanced Raman scattering.

In general, it can be desirable to perform the linking procedure sequentially, wherein the first step comprises adding linker molecules to individual particles under conditions that do not permit cross-linking of particles together. By way of example only, such a linker can comprise an oligonucleotide having a reactive group at one end only. During this first step, the reactive end of the oligonucleotide can bind with a metal particle, thereby forming a first particle-linker species, and having a free end of the linker. The ratio of linker molecules to particles can be selected, depending on the number of linker molecules are to be attached to the particle. A second linker can be attached to another group of particles in a different reaction chamber, thereby resulting in a second linker-particle species, again with the linker having a free end.

After those reactions have progressed, the different linker-particle species can be mixed together and the linkers can attach together to form "particle pairs" joined by the linker molecules.

II. Design and Manufacture of MDR Devices

The MDR-based device for enhancement comprises at least one microcavity providing morphology dependent resonance conditions placed in proximity to the surface of a substrate. Figure1 is a schematic diagram of a

system for the fluorescence and/or Raman detection of binding on a biochip embraced inside an MDR device **1**. This combined detection of both fluorescence and Raman spectra is useful for extensive spectroscopic characterization of an analyte in the presence or absence of a receptor. Such combined detection is used herein for illustrative purposes only, whereas detection of only fluorescence or only Raman signal is also possible using such a system, but using only one light source and one detection system.

The system shown on Figure 1 comprises two light sources **2** and **3**. The light source **2** can be for fluorescence measurements, and light source **3** can be for Raman measurements. The system can also include band pass filter **4**, mirrors **5** and **6**, polarizer **7** with variable phase plate, substrate **8** with sample **9**, optical fiber **10** for transmitting optical energy from the energy sources **2** or **3** to microcavity **11**. The light collector comprises an optic fiber **13** having a cavity **12** at one end for the embracement of the microcavity, collection optics **14**, a Raman holographic filter **15**, and an optical fiber **16** for transmitting the fluorescence signal. For Raman detection, an optical fiber **17** for transmitting the Raman signal, the fiber collector **18**, which can transmit signals to either the Raman spectrometer or to the fluorescence spectrometer. Coupling optics **19** provide the collecting of the fluorescence optical signal, and transmits this signal to a fluorescent signal analyzer **21**, and data processor **23** provides for the analysis of the fluorescence signal. Coupling optic **20**, signal analyzer **22** and data processor **24** provide for the analysis of the Raman signal from sample **9**. The light collector **1** is positioned so as to transmit the light signal from a defined biochip surface area to a defined areas of a CCD or photo-diode analyzer, which provides analyses of the signal light in parallel or consecutively, without scanning the surface of the biochip.

Figure 2 shows the "external" collecting system of this invention with a cylindrical cavity at the tip of an optic fiber. This system comprises a

microcavity **25**, an optic fiber **26** for transmitting optical energy into microcavity, substrate **27** with sample **28**, the optical fiber **29** with the cavity **12** for collecting optical fluorescence or Raman signal, and an opaque cover **31** on the external surface of the optic fiber. A soft rubber tip **30** can be used to avoid mechanical damage of the biochip surface upon positioning of the optic fiber **29** onto the biochip surface. Arrows **32** show signal transmitting direction.

Figure3 shows the "inner" collecting system. The light collector is an optic fiber inserted into a microcavity. This system comprises the microcavity **25**, optic fiber **26** for transmitting optical energy into the microcavity, a substrate **27** with a sample **28**, an optic fiber **33** (with opaque cover on the external surface of this optic fiber (**34**)) inserted in the microcavity. Arrow **32** shows signal transmitting direction. The external surface of the microcavity has mirror **35**, whereas the end of the microcavity **36** can be either opaque or mirrored to avoid loss of signal inside the light path and to avoid penetration of external, parasite signals from outside.

Figures 4a-4d show different types of analyte compartment arrangements according to certain embodiments of this invention. An analyte is bound to receptor **29** which is associated with planar surface **37**, or convex **27**, or hemispheric surface **39**, or to a particle structure **28**.

Figures 5a-5c depict alternative embodiments of low density arrays of this invention. Figure 5a depicts a top view of a device for MDR. Hemispheres **39** are shown within cylindrical cavity **29**. Incident light channel **29a** is shown attached to the wall of cylindrical cavity **29**. Hemispheres **39** can have particle structures **40** on their surface. Figure 5b depicts a cross-section along line A-A' of the device shown in Figure 5a. In Figure 5b, receptors **38** are shown on hemispheres **39** that are placed inside cylindrical cavity **29**. Particle structures **40** are shown on hemispheres **39**. Figure 5c depicts an embodiment of low

density array of this invention having 7 hexagonally arranged hemispheres **39** having receptors **38** thereon, within cavity **29**.

Figure 6a-6c depict two alternative embodiments of this invention of low density linear arrays inside microcavities. Figure 6a shows cylindrical cavity **29** having incident light channel **29a** attached. Detector **42** is shown associated with cavity **29**. Rectangular array **39** has a receptor **38** attached, and curved array **41** is shown having receptor **38** thereon. The arrays **39** or **41** can have different lengths between analytes **38a** (Figure 6b, **h1**, **h2**). Figure 6c depicts the surface of curved array **41** covered with particle structures **43** for additional enhancement of Raman or fluorescence signal from sample **38a**.

Figures 7a-7c depict alternative embodiments of this invention. Figure 7a shows conical optic fiber **44** for transmitting a signal from an analyte to a detector (not shown). conical tip permits adjustment of the distance (**h1** var)between the interior surface of cavity **48a** and fiber **44**. Optic fibers **49** and **50** are deliver incident light, and are shown in positions to illuminate a portion of cavity **48a**, which permits enhancement of signals from analytes **46**. Mirror **45** reflects the light signal towards detector. The surfaces **46** of the optical fiber can be covered with a nanoparticle structure **47**.

Figure 7b depicts an alternative embodiment of cavity **48**, but wherein the dimensions of fibers **49** and **50** and the wall **48** of the cavity are such that incident light illuminates all of the interior of cavity **48a**. The surfaces **46** of the optical fiber can be covered with a nanoparticle structure **47**.

Figure 7c depicts an embodiment of this invention otherwise similar to those embodiments depicted in Figures 7a and 7b except that there is no fiber for transmission of signal to a detector. Rather, signals are transmitted using other means (not shown). The device shown in Figure 7c can be readily combined with the detection system of Figure 2. The surfaces **46** of the optical fiber can be covered with a nanoparticle structure **47**.

III. Detection of Heterogeneous Analytes under MDR Conditions Using Addressable Arrays

Another aspect of this invention disclosed herein is a method, based on surface enhanced Raman scattering (SERS), for parallel direct detection of heterogeneous analytes upon simultaneous separation in microcavities using an addressable array of beads bearing target molecules. The method allows for a high throughput determination in mixtures of the amount of biochemically/structurally differing species, such as proteins, oligonucleotides, low-molecular weight molecules, etc., without their labeling. This quantitative determination of concentration of heterogeneous analytes present in a tissue sample, tissue or cell culture, bacterial suspension and so on, can be achieved using three principal steps: 1) simultaneous extraction of all desired analytes from a homogenate using specially prepared beads. The surface of each bead is covered with a very large number of a recognition molecules, so all molecules of a corresponding analyte can bind the receptor molecules on the beads' surfaces. Each bead also has a distinctive color or other physical feature, which can be recognized using Raman scattering, fluorescence or other techniques; 2) physical separation of the beads with bound analytes from unbound analytes can be accomplished by placing one bead in one tube. A distinctive color of each bead puts in a correspondence a tube and an analyte to be detected in it. Thus, the beads in separate tubes form an addressable array (which can be randomly differing from analysis to analysis), and 3) the beads in all tubes can be washed thoroughly, so that only specifically bound molecules are retained on the surface of the bead. Then, binding of analytes to their correspondent binding molecules can be disrupted by addition of a denaturant to all tubes (or corresponding denaturant to correspondent tubes). The beads can be thus removed from all tubes (for example, using a magnet for paramagnetic beads), whereas the

analytes remain in the denaturing solution in the tubes. The content of each analyte can be detected by means of Raman scattering or fluorescence under surface-enhanced conditions combined with MDR-enhancement. The surface-enhanced conditions are achieved in the presence of specially prepared particle structures either precast or generated prior to measurements. Alternatively, if there is a distinctive spectral feature which makes an analyte detectable in the presence of bead, no treatment with the denaturant and removal of the bead is performed.

Figures 8a and 8b show an arrangement for microwells **51** in a randomly addressable array **57** of this invention. Figure 8a depicts a cross-section of an array of this invention showing substrate **57a** having optical fibers **44** passing therethrough. The ends of fibers **44** are shown rounded and within microcavities **51**. Incident light channels **50** are shown in the wall **52** surrounding microcavities **51**. Small beads **53** are shown in the interior of cavities **51** In one microcavity, large bead **53a** is shown having particle structure **54** on the surface. Receptors **54a** are shown attached to the surface of particle structures **54**.

Figures 9a-9c shows an external detection system of this invention. Figure 9a depicts two microcavity structures **25** having microcavities **29** arranged in an array. Each structure has walls **25** surrounding microcavity **29**, optical channel **26**, a hemispheric surface **39**, bead **53**, external optical fiber **31** surrounding structure **25** for transmitting signal to detector (not shown). The tip of fiber **31** has a soft rubber layer **30**. Space **29a** is provided to permit lowering and raising fiber **31** without disturbing structure **25**.. The diameter of the optic channel **26** is larger than the diameter of the bead **53** to provide complete illumination of the bead. The hemispheric surface **39** can be used to position of the bead **53** in proximity to the inner wall of microcavity **25** for better MDR in microcavities **25** and beads **53**.

In general, beads used with the devices of this invention can be spherical, ellipsoidal, or have any regular shape, including, but not limited to cubic, faceted, geodesic, etc. Alternatively, beads can be polymeric and can have irregular shapes. In certain embodiments, beads can desirably be at least partially transparent to the wavelengths of electromagnetic radiation, and be capable of producing MDR conditions.

Figure 9b depicts two embodiments of bead **53**, **53a** and **53b**. Bead **53a** has particle structure **53c**, receptors **53d** and analyte **53e** retained by receptor **53d**. Bean **53b** does not have particle structure **53c** but has receptor **53d** with analyte **53e** retained thereby.

Figure 9c depicts a matrix array of several structures **25** arrayed on substrate **57a**.

Figures 10a-10b depict two embodiments of high density, addressable arrays of this invention, each having a wall **25** of microcavity **29**, an optic channel **26**, beads **53**, microwell **51** adapted to support beads **53**, each having optic fibers **44** for transmitting the signal from beads to the detector (not shown).

Figure 10b depicts an embodiment as in Figure 10a, except that holder **44a** can be used to simultaneously arrange an optical bundle of fibers **44** and to restrict the area of illumination by optical channel **26**.

IV. Non-planar arrays

Typically, arrays can be arranged on a planar surface. However, devices utilizing non-planar arrangement can have distinctive advantageous. First, the array can be embraced easily into a microcavity to detect analytes under MDR conditions. Next, MDR conditions and SERS conditions are readily combined on non-planar arrays, and finally, parasite light signals from neighboring sample compartments can be shielded due to the curved geometry. In one embodiment,

a cylindrical arrangement is described herein as an example of non-planar organization, whereas hemispheric or other non-planar arrangements can be also used. Non-planar arrays can be generated either at high density or low density of sample compartments.

Figures 11a-11b depict microcylindrical array **58** with optical fibers **62** leading to sample compartments **58a**. Optical fibers **62** are shown approaching compartments **58a** from the inside of array **58**. Two detailed arrangements for sample compartments **58a** are depicted in Figure 11b. In one embodiment **59c**, sample compartment **58a** contains only receptor **64** on concave surface **65** of optical fiber **62**. In another embodiment **58d**, a particle structure **67** supports bead **53** having receptors **54**..

Figure12 shows the cylindrical array **58** inside walls **59** of cavity **29**. Several optic channels **60** illuminate cavity **29**. A bundle of optic fibers **62** extend from the inside of array **58** transmit signal to an array of detectors (not shown).

Figure13a-13b depict shows a cylindrical array **63** inside a cylinder **66**, with a detection system comprising radial optic fibers **67** for transmission signals from microwells **65** to detectors (not shown). The transmitter of the optic energy can be the optic fiber **68**, as shown in Figure 13a, or waveguide **68a** arranged as shown in Figure 13b. Light is shown exiting waveguide **68a** (arrows).

Figures14a-14b depict a cylindrical array of this invention. Figure 14a shows array **63** having microwells **65** and optical channel **66** for illumination.

Figure 14b shows three microcavities **65** Microwells **65** are shown embedded into the surface of the microcylinder **63**. Microwells **65** of the array **63** are shown adjacent to optical channels **68**. Receptor **65a** is positioned in the left microwell without either particle structures or analytes. In the middle microcavity **67a**, particle structures **67**, receptor **65a** and analyte **65b** are shown.

In the rightmost microcavity, particle structures **67** are shown with analyte **65b** but without receptors. A bundle of the optic fibers **67a** which are in contact with microwells **65** is also shown. These fibers **67a** are placed adjacent to microwells **65**. The diameter of the optic fiber **67a** is larger than the diameter of the microwells **65**.

These embodiments are described for illustrative purposes only, and are not intended to limit the scope of this invention. Other embodiments of the invention can be designed and manufactured without undue experimentation, and are considered to be part of this invention.

INDUSTRIAL APPLICABILITY

The microcavities, analytes and detection apparatus of this invention are useful for the detection of analytes under conditions of morphology dependent resonance. Thus, the methods and apparatus of this invention can be advantageously used to improve the sensitivity and accuracy of many different types of analytical methods.

We claim:

1. An analyte detector comprising:
a microcavity;
a receptor associated with said microcavity;
a source of electromagnetic radiation; and
a detector.

2. The analyte detector of claim 1, further comprising an analyte associated with said receptor.

3. The analyte detector of any of claims 1 - 2, further comprising a particle structure.

4. The analyte detector of claim 2, further comprising a particle structure.

5. The analyte detector of any of claims 1 - 3, wherein said microcavity has an interior shape selected from the group consisting of cylindrical, conical, spherical, ellipsoidal and regular polygonal.

6. The analyte detector of claim 5, wherein said regular polygonal shape is selected from the group consisting of triangular, quadralateral, pentagonal, hexagonal, heptagonal, octagonal, nonagonal and decagonal.

7. The analyte detector of any of claims 1 - 6, further comprising a bead associated with said receptor.

8. The analyte detector of any of claims 1 - 7, further comprising a bead associated with said analyte and said receptor.

9. The analyte detector of claim 7, wherein said bead is selected from the group of shapes consisting of spherical, ellipsoidal and regular solids.

10. The analyte detector of any of claims 7 - 9, wherein said bead permits at least a portion of incident electromagnetic radiation to enter said bead.

11. The analyte detector of claim 1, wherein said particle structure is a fractal.

12. The analyte detector of claim 2, wherein said particle structure is a fractal.

13. The analyte detector of claim 7, further comprising a particle structure.

14. The analyte detector of claim 8, further comprising a particle structure.

15. The analyte detector of any of claims 1 - 14, wherein said source of electromagnetic radiation produces monochromatic radiation.

16. The analyte detector of any of claims 1 - 15, wherein said source of electromagnetic radiation produces a mixture of wavelengths of radiation.

17. The analyte detector of any of claims 1 - 16, further comprising a plurality of sources of electromagnetic radiation.

18. An device for detection of analytes, comprising:

a plurality of microcavities, at least one of which contains a receptor;

an analyte associated with said at least one receptor;

a source of electromagnetic radiation; and

a detector capable of independently detecting each of said plurality of microcavities.

19. The device of claim 18, wherein at least a portion of said plurality of microcavities is associated with a different receptor.

20. An array for analyte detection, comprising:

a matrix having a plurality of defined regions with microcavities therein; at least one region associated with an independent source of electromagnetic radiation and a detector.

21. The array of claim 20, wherein said defined areas are positionally defined.

22. The array of any of claims 20 - 21, wherein said defined areas are defined using a unique feature.

23. The array of any of claims 20 - 22, wherein said defined areas are defined using a unique feature of a bead.

24. The array of any of claims 20 - 23, wherein said matrix is planar.

25. The array of any of claims 20 - 23, wherein said matrix is curved, having a convex surface and a concave surface.

26. The array of claim 25, wherein said matrix is cylindrical

27. The array of claim 25, wherein said microcavities are on the convex surface of said matrix.

28. The array of any of claims 20 - 27, wherein said microcavities comprise a particle structure.

29. The array of claim 28, wherein said particle structure is a fractal structure.

30. A system for detecting analytes, comprising:

a matrix having an array of microcavities thereon, a plurality of said microcavities, at least one being associated with a receptor;

a source of electromagnetic radiation;

a detector; and

a computer for analyzing detected electromagnetic radiation.

31. The system of claim 30, further comprising a memory device for storing a program for analyzing said detected electromagnetic radiation.

32. The system of any of claims 30 - 31, further comprising beads having receptors thereon.

33. The system of any of claims 30 - 32, further comprising beads having receptors thereon and a particle structure associated with at least one of said microcavities.

34. The system of any of claims 30 - 33, further comprising an input device for retrieving information from a database.

35. The system of any of claims 30 - 34, further comprising an output device for transmitting information to a database.

36. A method for detecting analytes, comprising the steps of:

providing a system comprising:

a matrix having an array of microcavities thereon, a plurality of said microcavities, at least one being associated with a receptor;

a source of electromagnetic radiation;

a detector; and

a computer for analyzing detected electromagnetic radiation;

providing an analyte

placing said analyte in proximity to said at least one receptor;

exposing said analyte to incident electromagnetic radiation; and

detecting an electromagnetic signal associated with said analyte.

37. The method of claim 36, further comprising the step of:

comparing said electromagnetic signal with a signal associated with a known moiety.

38. The method of any of claims 36 - 37, further comprising the step of:

depositing data associated with the signal associated with said analyte in a database.

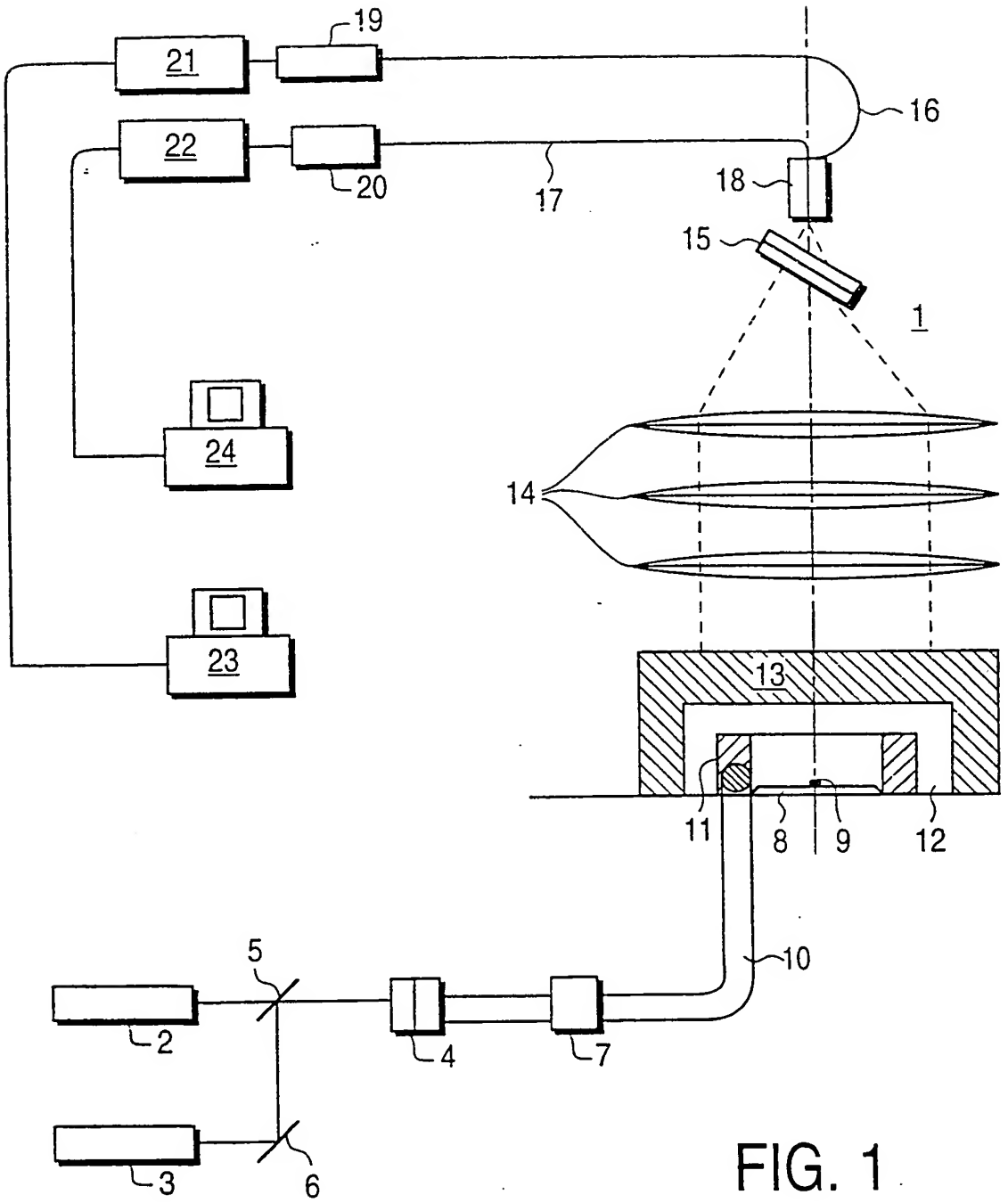


FIG. 1

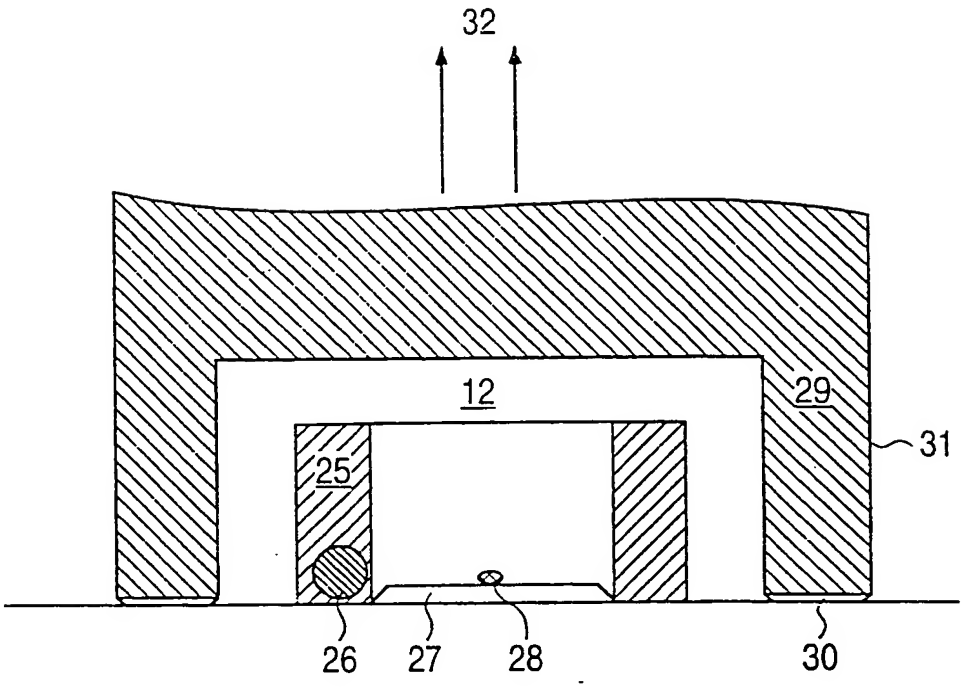


FIG. 2

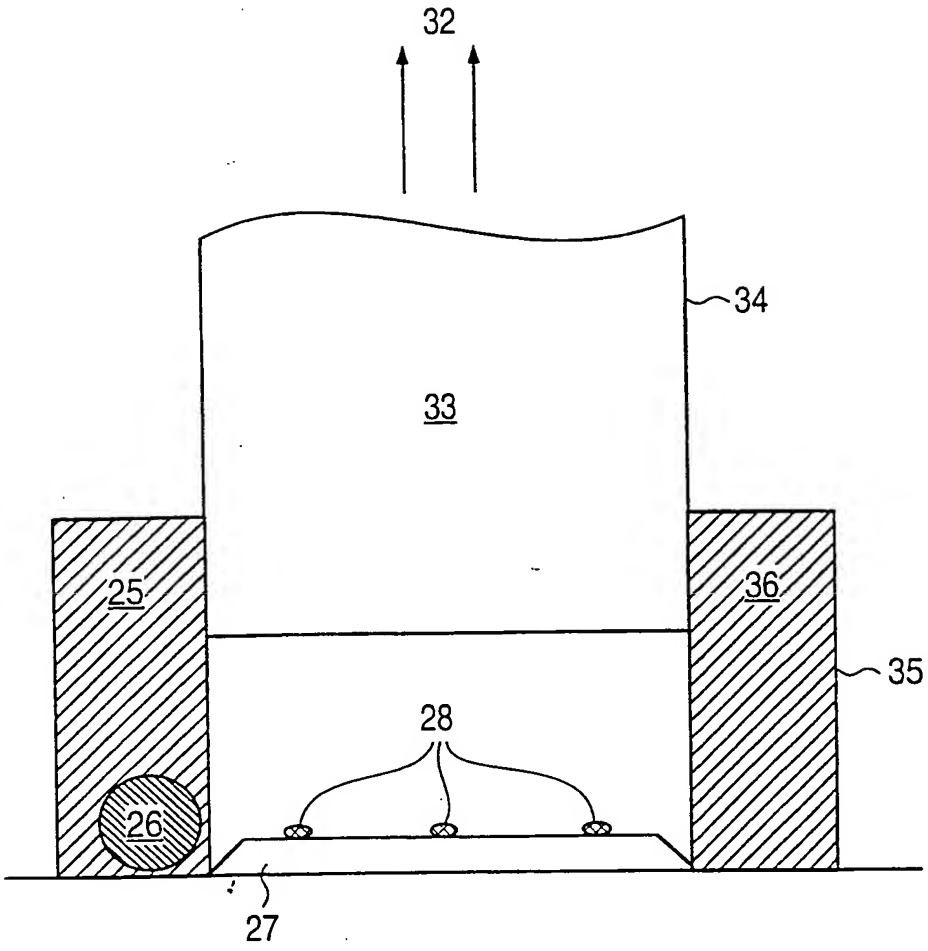


FIG. 3

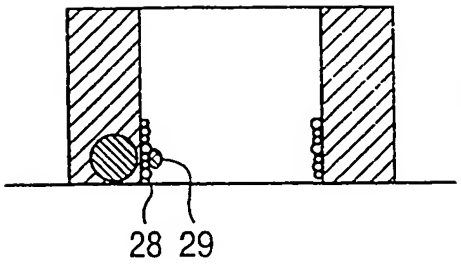
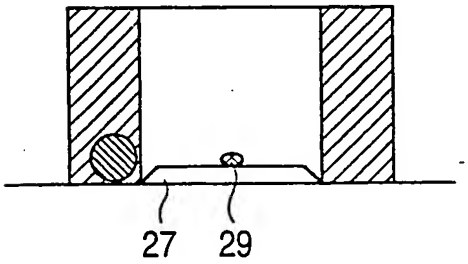


FIG. 4a

FIG. 4b



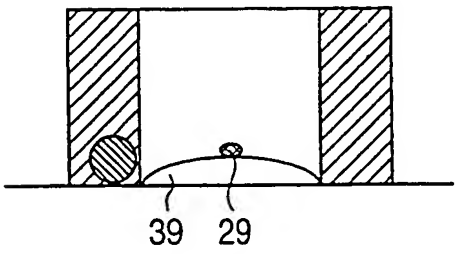
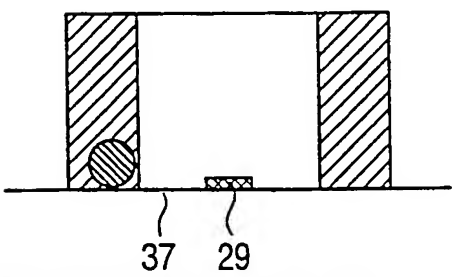


FIG. 4c

FIG. 4d



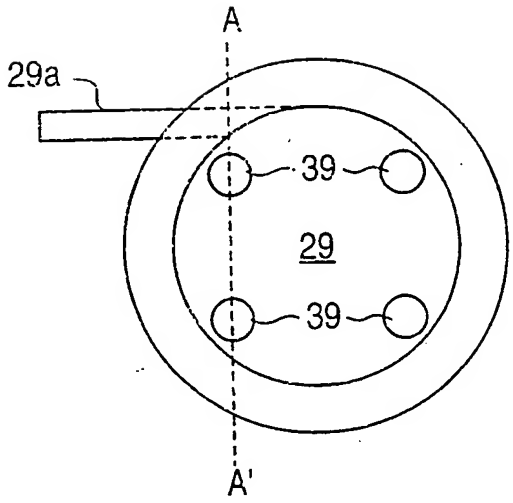
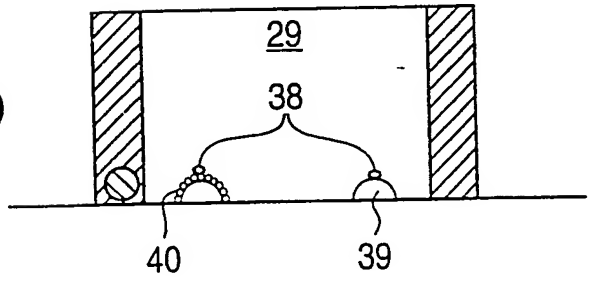


FIG. 5a

FIG. 5b



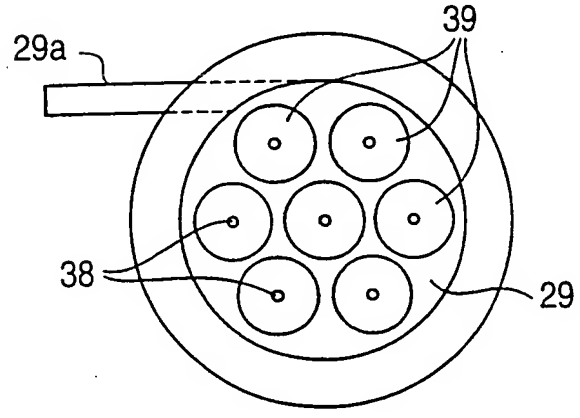


FIG. 5c

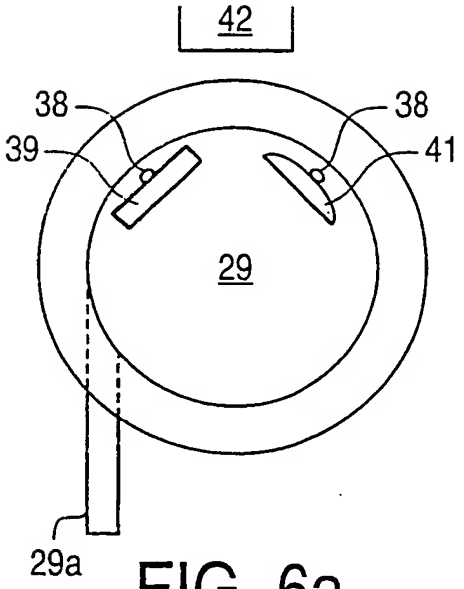


FIG. 6a

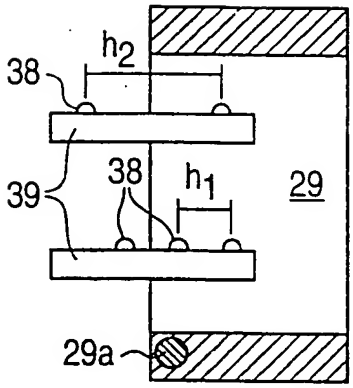


FIG. 6b

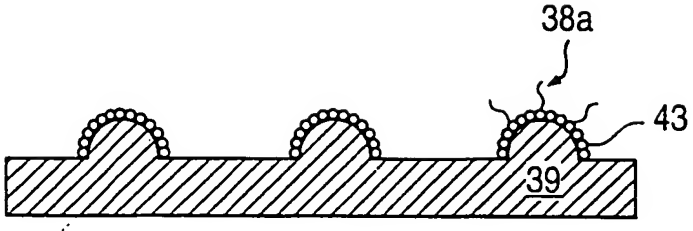


FIG. 6c

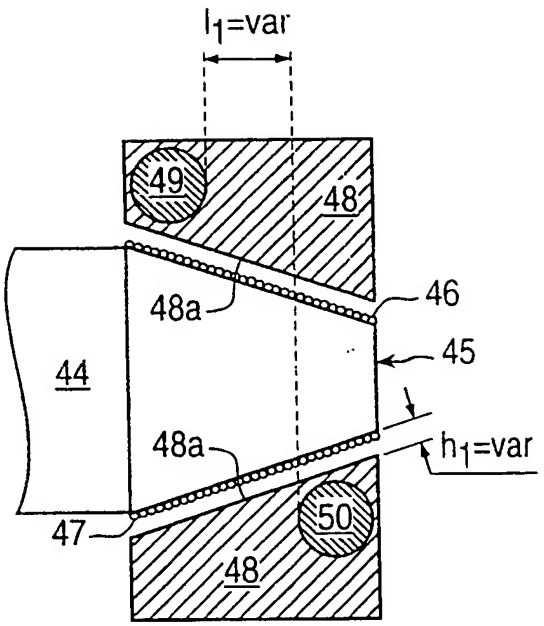


FIG. 7a

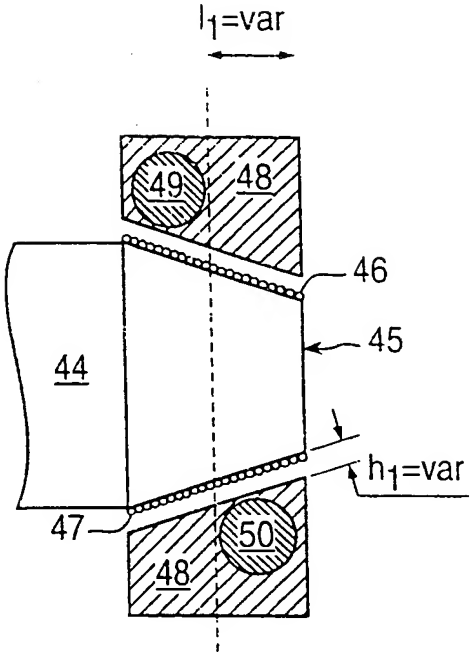


FIG. 7b

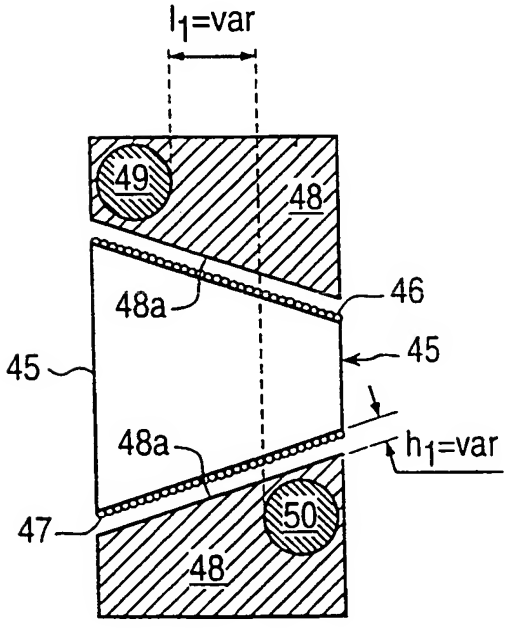


FIG. 7c

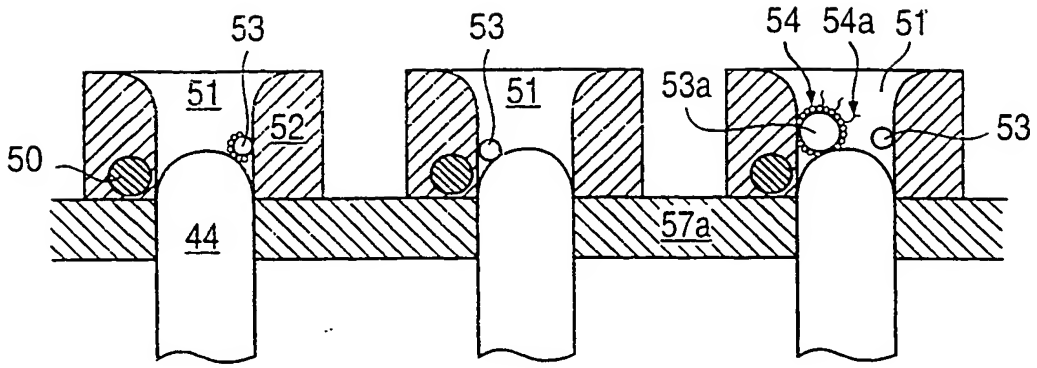


FIG. 8a

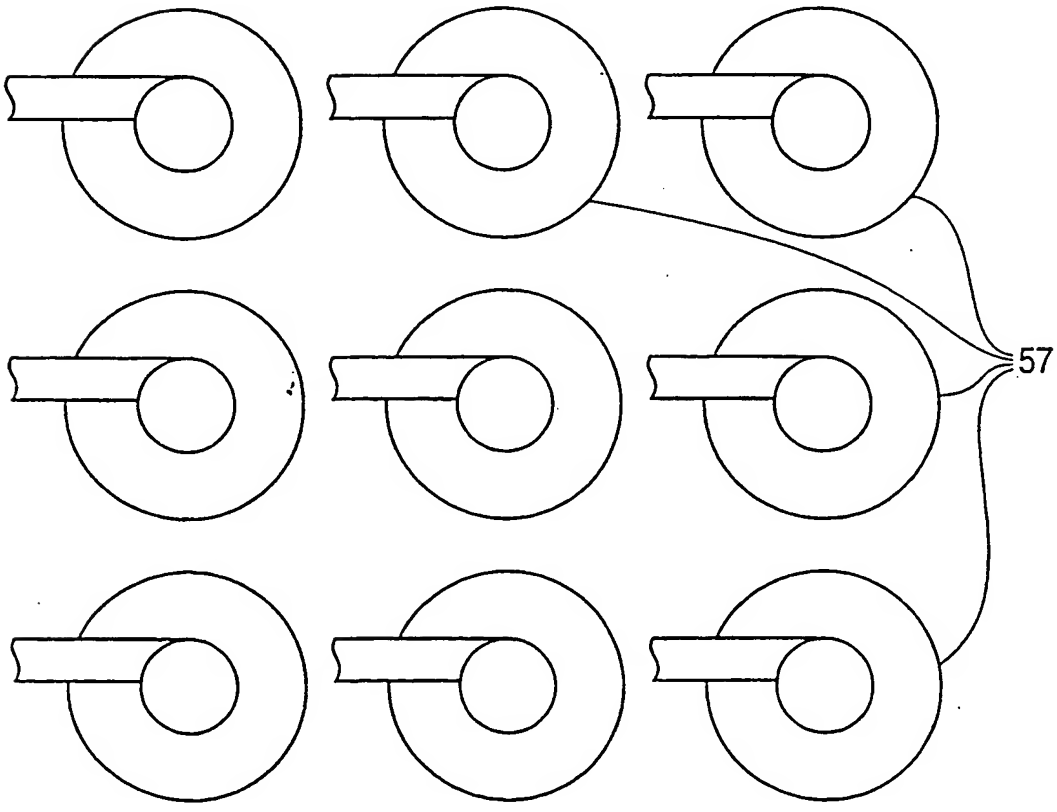


FIG. 8b

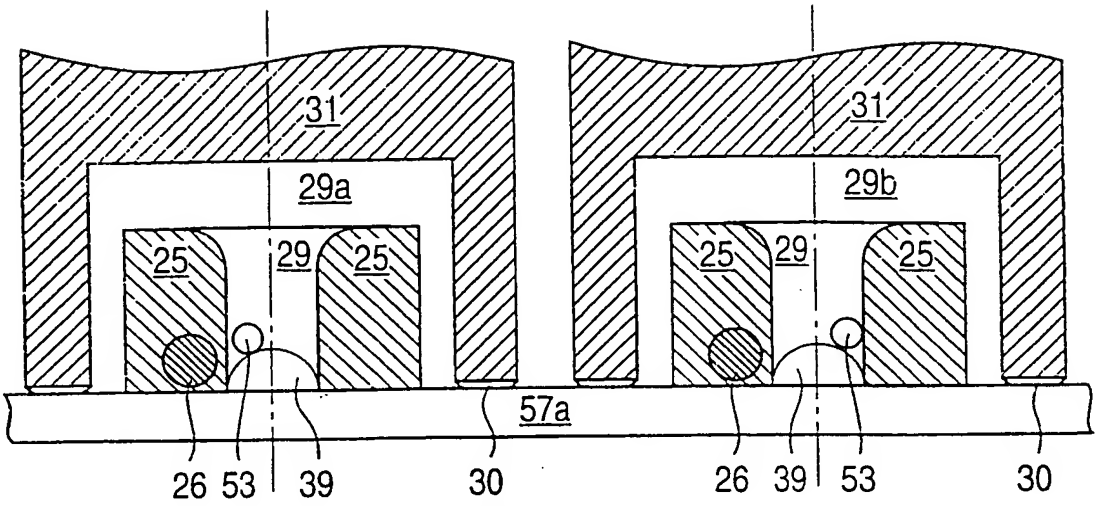


FIG. 9a

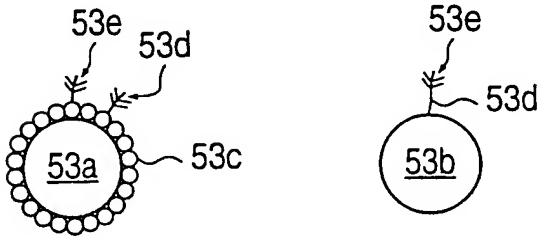


FIG. 9b

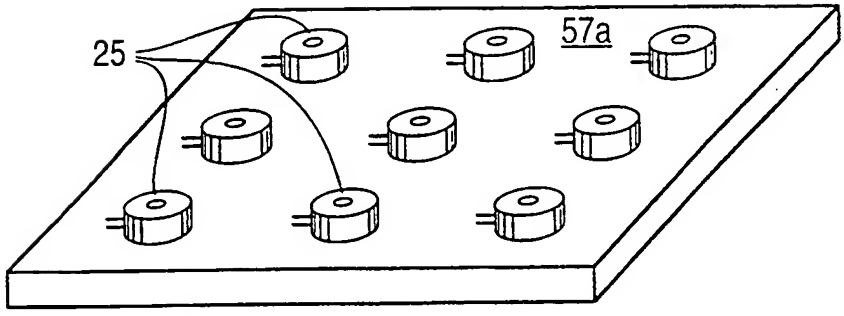


FIG. 9c

FIG. 10a

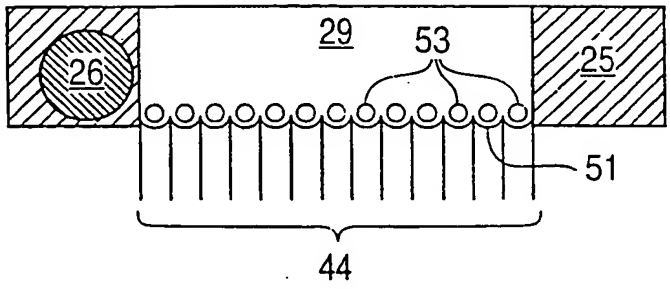
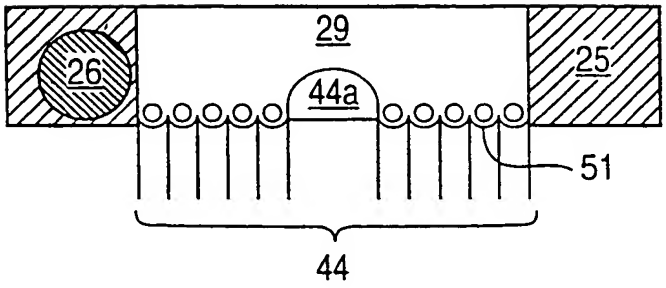


FIG. 10b



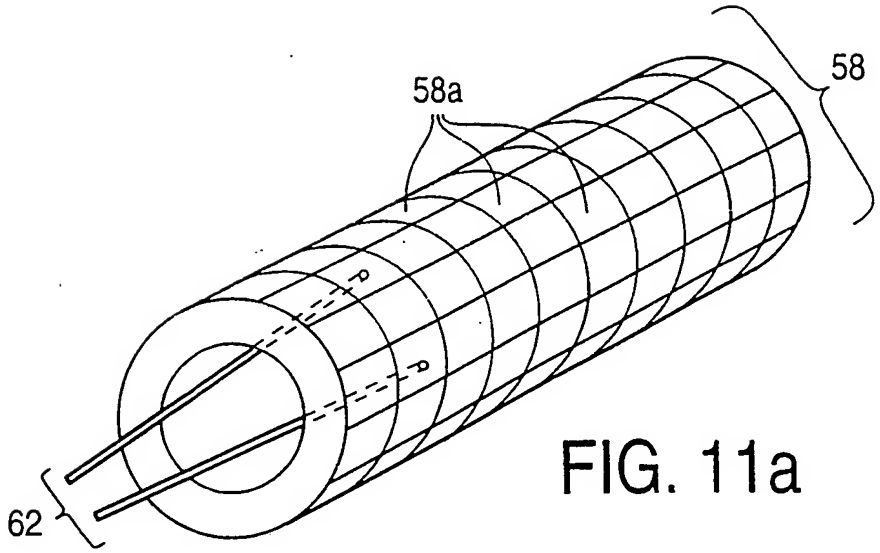


FIG. 11a

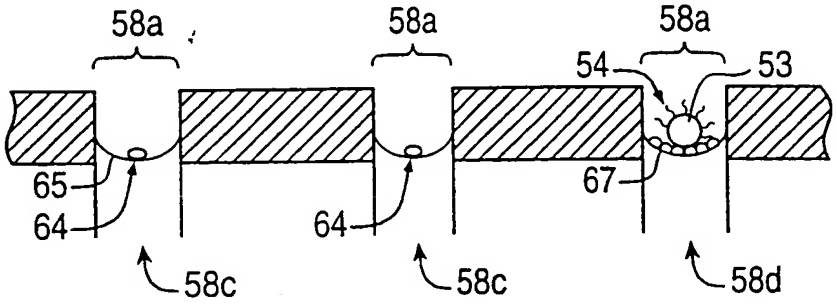


FIG. 11b

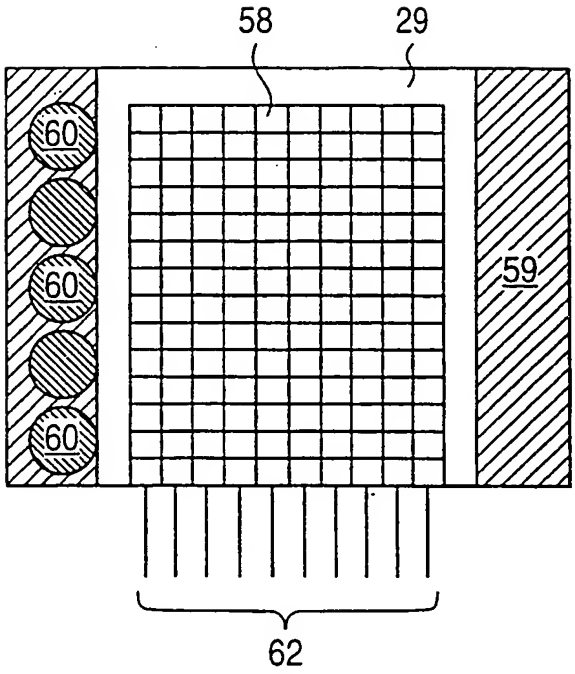


FIG. 12

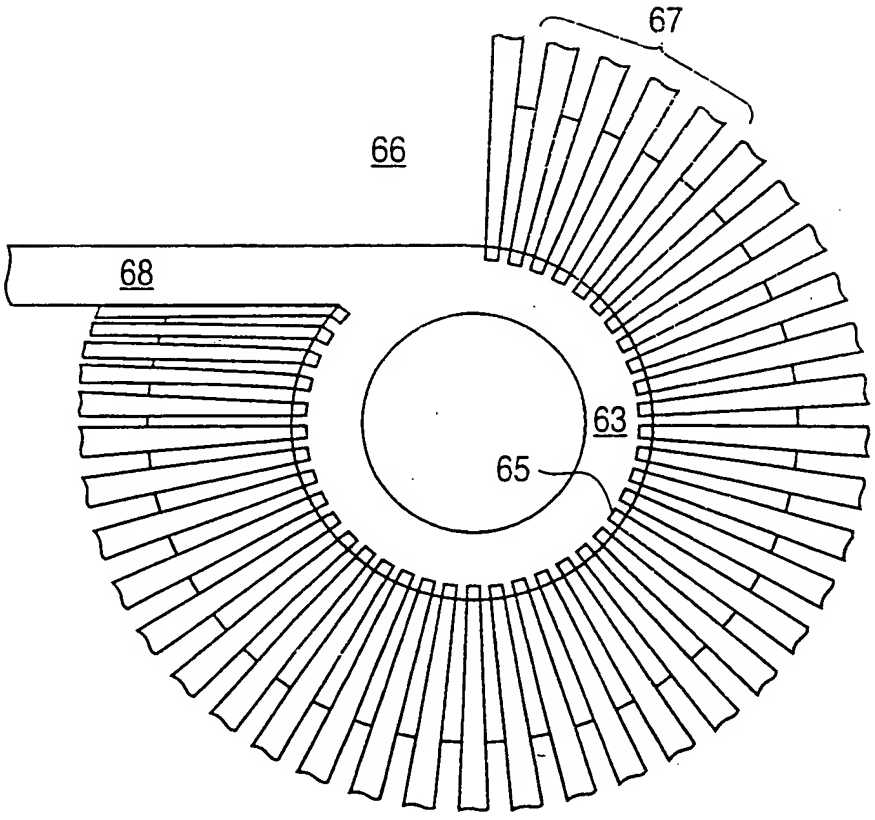


FIG. 13a

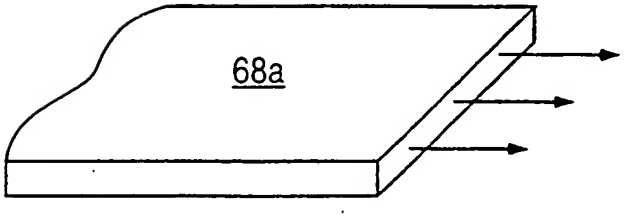


FIG. 13b

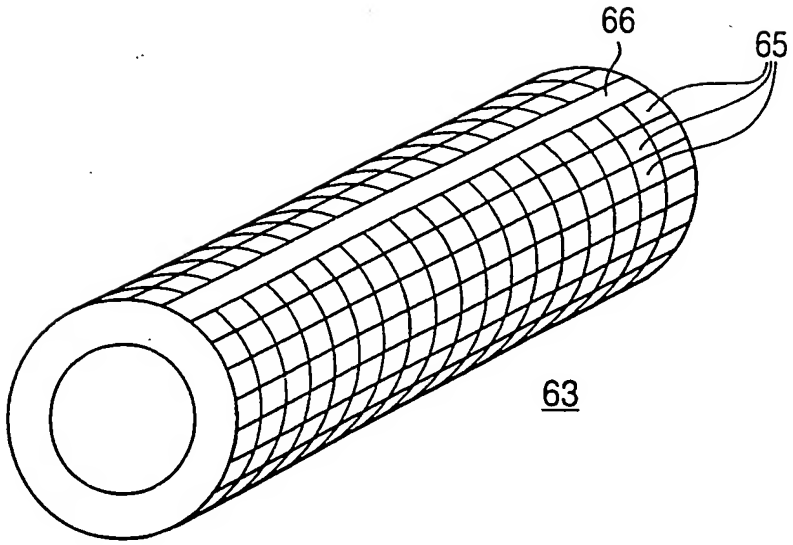


FIG. 14a

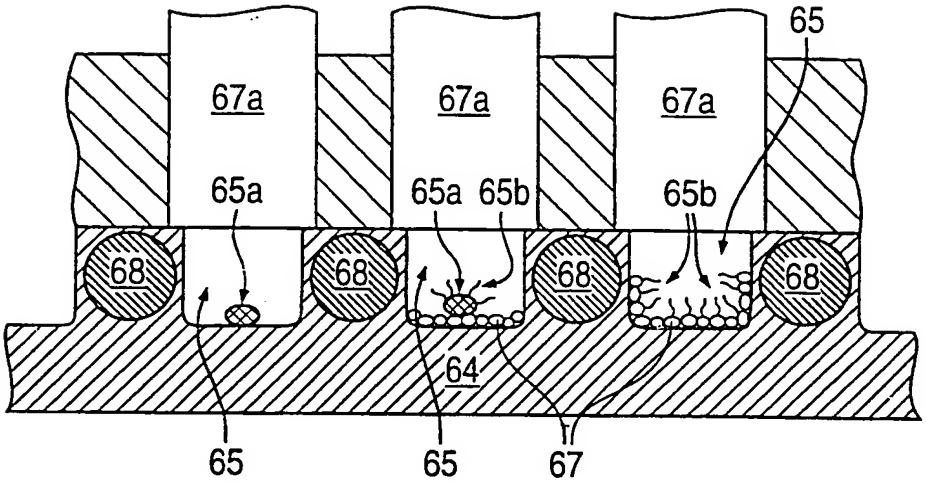


FIG. 14b

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US00/26413

A. CLASSIFICATION OF SUBJECT MATTER

IPC(7) :G01N 33/53
US CL :422/82.05

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 422/82.05-82.11,55; 436/164,171,805

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

none

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

none

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US 5,814,565 A (REICHERT et al) 29 September 1998, see the abstract, figure 5 and column 5	1-38

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E"	earlier document published on or after the international filing date	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&"	document member of the same patent family
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search 21 DECEMBER 2000	Date of mailing of the international search report 22 JAN 2001
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703) 305-3230	Authorized officer LYLE A. ALEXANDER Jean Proctor Paralegal Specialist Telephone No. (703) 308-0661

Form PCT/ISA/210 (second sheet) (July 1998)*